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Physicochemical properties of alginate-based films: Effect of ionic crosslinking and mannuronic and guluronic acid ratio

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ABSTRACT

The use of alginates as films in food applications has increased in the recent years due to their swelling capacity and overall functionality. This behaviour is a result of their capacity to crosslink with Ca^{2+} ion. Aiming to fully understand the effect of calcium chloride ($CaCl_2$) crosslinking and the mannuronic (M) and guluronic (G) acid ratio (M/G) of alginate structure in the films' properties, alginate-based films with different (M/G) ratios were crosslinked at increasing $CaCl_2$ concentrations. Films were produced by casting, and characterized in terms of mechanical properties (tensile strength and elongation-at-break), opacity, water sensitivity (moisture content, solubility and water vapour permeability) and morphology, evaluated by scanning electronic microscopy (SEM). Chemical interactions were studied by Fourier Transform Infrared Spectroscopy (FTIR) to assess possible chemical modifications of alginate-based films after crosslinking. Crosslinking significantly affected the alginate structure and properties, decreasing film thickness, moisture content, solubility and water vapour permeability of the alginate-based films. The mechanical properties were also influenced by the crosslinking and high $CaCl_2$ concentrations lead to an increase of tensile strength. Results showed a relation between M/G ratios and $CaCl_2$ concentrations and the resulting film's properties. Alginate and the respective crosslinker should be chosen taking into account M/G ratio, since high contents of M residues lead to fragile and flexible films and high content of G residues to stronger films, and these properties are highly dependent on the concentration of $CaCl_2$. Overall, alginate-based films are a good candidate to obtain tailored made edible films for food applications. Further investigation should be done to fully understand the effect of the alginate chain composition and order (e.g. MM, GG, GM, MG) in alginate-based films properties.

1. Introduction

Edible films experienced a notable growth in recent years, presenting nowadays an important impact in the food industry. This growth is due to the increasing interest on new materials using bio-based and biodegradable polymers to replace the non-biodegradable and petroleum-based ones, but also for the development of new and innovative food products (Cerqueira, Teixeira, & Vicente, 2016; Cerqueira et al., 2011). In this context edible films and coatings appeared as some of the materials with great interest. They can be produced by different edible materials such as: polysaccharides, protein, and lipids, with the possible addition of plasticizers and/or surfactants (Dhanapal et al., 2012), their performance being directly related with their chemical characteristics, production method and environmental conditions where they are used.

One of the most interesting polysaccharides for the development of these structures is alginate. It presents non-toxic and unique colloidal properties, such as: thickening, stabilizing, suspending, film forming, gel producing and emulsion stabilizing (Dhanapal et al., 2012). Films formed by alginate are uniform, transparent and good oxygen barriers but have poor water resistance because of their hydrophilic nature (Dhanapal et al., 2012; Lin & Zhao, 2007; Rhim, 2004). Alginates are hydrophilic colloidal carbohydrates extracted from various species of brown seaweeds, which belong to the *Phaeophyceae* class. They present a linear structure formed by sequences of α-(1–4)-linked units of β-D-

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mannuronic acid (M blocks) and α-L-guluronic acid (G blocks) residues in different proportions and with different distributions in the chain, being the carboxylic groups from uronic acids responsible for their negative charge. The molecular weight and sequence of the M and G residues affects the physicochemical properties of alginates, being the ratio of mannuronic and guluronic acid residues dependent on the biological source and maturation state of the seaweeds (Lee & Mooney, 2012; Milani & Maleki, 2012). Alginates have the ability to react with di- and trivalent cations, specifically calcium ions. Calcium-induced gelation has been demonstrated to result from specific and strong interactions between Ca^{2+} with G blocks of alginate, resulting in the "egg-box" structure (Nieto, 2016; Rhim, 2004; Yoon, Oh, Jo, Lee, & Hwang, 2014) represented in Fig. 1.

There are different steps occurring for the "egg-box" formation, relying first on the interaction between the calcium ion and the G monomer, secondly in the formation of egg-box dimers and at last in the association of dimers resulting in multimers (Fu et al., 2011). The predisposition to bind with Ca^{2+} occurs in G blocks. Some factors like the G sequence, G percentage and alginate molecular weight are very relevant to determine how the dimers and multimers will associate and influence the strength of the produced structure (Fu et al., 2011). Recently, several works showed that the crosslinking of alginate-based films with calcium leads to higher values of tensile strength, increasing the structure cohesion leading to stronger films with low solubility in water (Cathell & Schauer, 2007; Galus, Uchański, & Lenart, 2013; Liling et al., 2016; Nieto, 2016; Remuñán-López & Bodmeier, 1997; Russo, Malinconico, & Santagata, 2007; Ying, 2006, p. 1–77). However, to the best of the authors' knowledge, there are not works showing the influence of different M/G ratio in films' properties and how the crosslinking influences differently this type of structures. Based on this, the objectives of this work were to evaluate the effect of ionic crosslinking on physicochemical properties of alginate-based films with different mannuronic and guluronic acid ratio, and establish a relationship between their chemical characteristics and film's properties.

2. Materials and methods

2.1. Materials

Sodium alginate CR8223 (FMC BioPolymer) with M/G ratio of 65/35 and a molecular weight (MW) of 300 kDa, and Manugel FB (FMC BioPolymer) with M/G ratio 30/70 and a MW < 200 kDa were kindly provided by Eurosalmo (Portugal). Calcium chloride was obtained from Panreac (Spain) and Glycerol 99% was obtained from Himedia (India).

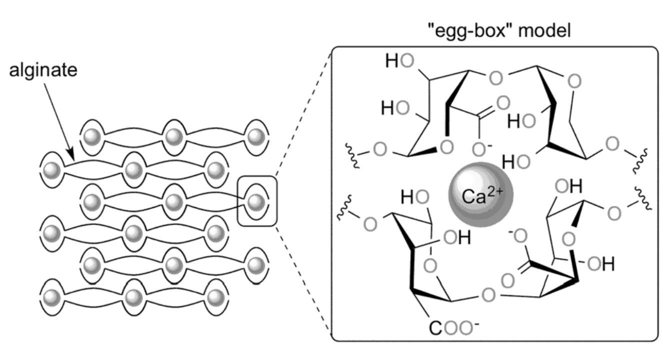

Fig. 1. Alginate crosslinking with Ca^{2+} and "egg box" formation [Kühbeck, D., Mayr, J., Häring, M., Hofmann, M., Quignard, F., & Díaz Díaz, D. (2015). Evaluation of the nitroaldol reaction in the presence of metal ion-crosslinked alginates. New J. Chem., 39(3), 2306–2315] - Published by The Royal Society of Chemistry (RSC) on behalf of the Centre National de la Recherche Scientifique (CNRS) and the RSC.

2.2. Production of the films

Film-forming solutions of alginate were prepared by dissolving alginate 1% (w/v) in distilled water under agitation (350 rpm) at room temperature for 18 h. After that, glycerol (0.5% w/v) was added and the solutions stirred (350 rpm) for 12 h at room temperature and homogeneous solution was obtained. The concentration of alginate and glycerol was chosen based on preliminary studies where the processability and the formation of homogeneous films were guaranteed (results not shown).

The film-forming solutions were cast in polystyrene petri plates and dried at 30 °C for 48 h. Films with thickness values ranged between 40 μm and 60 μm were obtained. Films were conditioned in desiccators containing a saturated solution of $Mg(NO_3)_2.6H_2O$ at 53% of relative humidity (RH) and 20 °C before analyses.

2.3. Crosslinking of the films

Solutions with different concentrations (1%, 1.25% and 1.5% w/v) of calcium chloride ($CaCl_2$) were used in the crosslinking (CL) process of CR8223 (CR) and Manugel FB (MG) films, as described by Rhim (2004) with some modifications. After 5 min of immersion (determined in preliminary studies aiming a homogeneous film) the excess of $CaCl_2$ solution was discarded and films (CR-CL and MG-CL) were left to dry at room temperature during 16 h. Films were conditioned in desiccators containing a saturated solution of $Mg(NO_3)_2.6H_2O$ at 53% relative humidity (RH) and 20 °C before analysis.

2.4. Moisture content and water solubility

To determine the films moisture content (MC), the method described by Costa et al. (2015) was used. Briefly, films with 2 cm of diameter were dried at 105 °C during 24 h (until the equilibrium weight was achieved). The sample weight loss was determined, and MC was calculated as the percentage of water removed from the system. Afterwards the film solubility in water was determined. The dried films were immersed in 50 mL of water and after 24 h of immersion at 20 °C with agitation (150 rpm), the insoluble pieces of film were taken out and dried to constant weight in an oven at 105 °C, to determine the weight of dry matter that was not solubilized in water.

2.5. Water vapour permeability (WVP)

Water vapour permeability (WVP) was based on the methodology described by Costa et al. (2015). Films were sealed on cups with distilled water and placed at 0% RH and 20 °C. Cups were periodical weighed (2 h) and weight loss was measured over time until steady state was reached. Water vapour transmission rate (WVTR) was calculated by dividing the slope of a linear regression of weight loss versus time by film area, and WVP ($g\ m^{-1}\ s^{-1}\ Pa^{-1}$) determined as follows in equation (1):

$$WVP = \frac{(WVTR \times L)}{\Delta P} \tag{1}$$

where L is the film thickness (m) and ΔP is the water vapour partial pressure difference (Pa) across the two sides of the film. For each measurement, at least three replicates were made for each alginate film sample.

2.6. Swelling index

The swelling index (SW) of films was determined as described by Cao, Fu, & He (2007) with some modifications. The films were cut in squares with $2 \times 2\,cm^2$ size and the weight was measured. Afterwards pre-weighed films were immersed in distilled water for 24 h at room temperature. Then, samples were wiped with filter paper to remove liquid excess and the final weight was measured. The amount of absorbed water in percentage was calculated using equation (2), where S_1 is the weight of the film after immersion and S_0 is the initial weight of the film. All measurements were performed in triplicate for each type of film.

$$SW = \frac{(S_1 - S_0)}{S_0} \times 100 \tag{2}$$

2.7. Opacity and colour

The opacity based on the CIELab was determined as described by Costa et al. (2015). The total difference value (ΔE) and the whiteness value (WI) was calculated according to equations (3) and (4) respectively. Ten measurements were performed for each film and the mean values were used to determine each parameter.

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{0.5} \tag{3}$$

$$WI = Y + (WI, x)(x_n - x) + (WI, y)(y_n - y) \tag{4}$$

Where $\Delta L = L_{standard}$-L_{sample}; $\Delta a = a_{standard}$-a_{sample}; $\Delta b = b_{standard}$-b_{sample}. Standard values used were of MG and CR films without crosslinking: $L = 96.33$, $a = 0.10$ and $b = 4.49$ for MG and $L = 95.05$, $a = -0.14$ and $b = 7.82$ for CR. ASTM E313 methodology was used to calculate WI where Y, x, y are the luminance factors and the chromaticity coordinates of the specimen and x_n and y_n are the chromaticity coordinates for the used CIE standard illuminant (0.3158 and 0.3321, respectively) and WI,x and WI,y are numerical coefficients (800 and 1700 respectively).

2.8. Scanning electron microscopy (SEM)

The surface morphologies of CR and MG films with and without crosslinking (CR and MG films) and with crosslinking (CR-CL and MG-CL films) were examined using scanning electron microscopy (SEM) (Quanta FEG 650, FEI, USA) with an accelerating voltage of 5 kV. Before analysis, all samples were mounted on aluminium stubs using carbon adhesive tape and sputter-coated with gold and then where shredded.

2.9. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the films was recorded with a Bruker FT-IR VERTEX 80/80v (Boston, USA) in Attenuated Total Reflectance mode (ATR) with a platinum crystal accessory in the wavenumber range: 4000-400 cm^{-1}, using 16 scans at a resolution of 4 cm^{-1}. Before analysis, an open bean background spectrum was recorded as a blank. All measurements were performed in triplicate.

2.10. Mechanical properties

The mechanical properties of the films were measured following the methodology described by ASTM D882-10 and using the texture analyser (TA.HD plus, Stable Micro Systems, United Kingdom) with the software Exponent. Samples (120 mm × 20 mm) were clamped between grips with an initial distance of 100 mm. The force and deformation were recorded during extension at 50 mm min^{-1}. Results of tensile strength (TS), Young's modulus (YM) and elongation-at-break (EB) were obtained in MPa and percentage respectively. Five replicates of each film were performed.

2.11. Statistical analysis

Statistical analysis was performed using the analysis of variance (ANOVA) procedure with Statistica software for Windows (free trial). Tukey's test was applied to detect differences of means, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Moisture content and water solubility

Moisture content (MC) was used to understand how the affinity of alginate-based films to water was influenced by the M/G ratio and the crosslinking with different $CaCl_2$ concentrations. Cross-linked films (both CR-CL and MG-CL films) presented lower values of MC showing that the water affinity in CR and MG alginate films is highly influenced by the crosslinking (Table 1). This behaviour is explained by the crosslinking process, in which the COO^- in the alginate binds to the Ca^{2+}, leading to the "egg-box" formation. Consequently, the alginate chains become less available to bind with H_2O molecules, since hydrophilic sites along alginate chains become less exposed resulting in lower values of MC (Rhim, Gennadios, Weller, Carole Cezeirat, & Hanna, 1998).

For CR-CL films, the increase of $CaCl_2$ concentrations did not affect significantly ($p > 0.05$) the MC values. However, looking for the MC values of MG-CL films, it is observed a decrease of the MC values for higher $CaCl_2$ concentrations (Table 1). This behaviour is

Table 1
Values of moisture content, solubility, swelling index and water vapour permeability (WVP) of CR, MG, CR-CL and MG-CL films crosslinked with 1%, 1.25% and 1.5% of $CaCl_2$.

Film	Moisture content (%)	Solubility (%)	Swelling index (%)	WVP x 10^{-11} (g m^{-1} s^{-1} Pa^{-1})
CR	$41.40 \pm 1.62^{A,a}$	$100.00 \pm 0.00^{A,a}$	N/A	$10.5 \pm 0.44^{A,a}$
CR-CL 1%	$22.20 \pm 4.11^{B,b}$	$51.88 \pm 3.80^{B,b}$	$695.94 \pm 101.37^{A,a}$	$3.71 \pm 0.34^{C,b}$
CR-CL 1.25%	$19.71 \pm 3.28^{C,b}$	$45.25 \pm 4.01^{D,c}$	$485.17 \pm 85.14^{B,b}$	$3.96 \pm 0.04^{E,b}$
CR-CL 1.5%	$20.73 \pm 1.78^{E,b}$	$31.14 \pm 4.59^{F,d}$	$411.55 \pm 32.44^{D,b}$	$3.73 \pm 0.27^{G,b}$
MG	$37.29 \pm 2.47^{A,a}$	$100.00 \pm 0.00^{A,a}$	N/A	$7.83 \pm 0.98^{B,a}$
MG-CL 1%	$26.12 \pm 3.42^{B,b}$	$77.72 \pm 11.82^{C,b}$	N/A	$5.93 \pm 0.38^{D,b}$
MG-CL 1.25%	$26.38 \pm 2.29^{D,b}$	$66.41 \pm 6.12^{E,b}$	$281.18 \pm 57.45^{C,a}$	$5.21 \pm 0.25^{F,b,c}$
MG-CL 1.5%	$21.78 \pm 2.02^{E,c}$	$49.58 \pm 7.20^{G,c}$	$209.99 \pm 69.24^{E,a}$	$4.82 \pm 0.09^{H,c}$

Values reported are the mean ± sd. Different letters (a–d) in the same column indicate a statistically significant difference ($p < 0.05$) between CR films or MG films and different letters (A-G) indicate a statistically significant difference ($p < 0.05$) between the CR and MG films for the same $CaCl_2$ concentrations.

related to the M/G ratio of the alginate. The Ca^{2+} ions mostly react with G block that must occur in series in the chain (e.g. GG or GGG) (Grant, Morris, Rees, Smith, & Thom, 1973). This implies that a high G content demands for high concentrations of $CaCl_2$ to bind all the G blocks and thus forming the “egg-box” structure. Being so, for the MG films, where the M/G ratio is 30/70, there is a need for higher concentrations of Ca^{2+} to form the “egg-box” structure. Nevertheless, for the CR and MG films crosslinked with the same concentration of $CaCl_2$ the obtained MC values did not present any statistical differences ($p > 0.05$), with exception of the films crosslinked with 1.25% of $CaCl_2$, where the MC values were 19.71 and 26.38% for the CR and MG films, respectively.

Regarding water solubility, the alginate-based films without crosslinking (CR and MG films) were completely soluble in water. On the other hand, the crosslinked alginate based-films (CR-CL and MG-CL) presented significant statistical differences when an increasing concentration of $CaCl_2$ (1%, 1.25% and 1.5% w/v) was used during the crosslinking process. The films crosslinked with higher concentrations of $CaCl_2$ presented the lowest values of solubility (Table 1). The fact that solubility is still decreasing with the increase concentration of $CaCl_2$ gives the indication that there are still free G blocks available in the matrix, able to form the “egg-box” structures. Another interesting result, is that for MG films the solubility was higher when compared with the results obtained for CR films crosslinked with the same $CaCl_2$ concentrations. Those observations confirm that a high number of G in the alginate structure results in films that need more Ca^{2+} to form a low soluble structure and therefore reach the same values of solubility than the films formed with an alginate with a low G content. Other important fact that can explain the high solubility values of crosslinked MG films, when compared with crosslinked CR films, is the alginate molecular weight (MW); alginate CR has a higher MW (300 kDa) than alginate MG (<200 kDa) and consequently a longer chain can lead to stronger films and thus low solubility values.

3.2. Water vapour permeability (WVP)

The evaluation of WVP of the films is important to understand how alginate films composition (i.e. M/G ratio) and crosslinking influence parameters like solubility and diffusion of water vapour molecules in the film matrix. Table 1 presents the WVP values of the studied films. The permeability of the films was significantly changed ($p < 0.05$) by the crosslinking process, resulting in films with lower WVP values. Some differences were observed for increasing concentrations of $CaCl_2$ according to alginate used. For CR-CL films the increase of $CaCl_2$ concentration did not influence WVP while for MG-CL films the use of higher concentrations of $CaCl_2$ (1.25 and 1.5%) led to lower WVP values. These results showed that the crosslinking process influences the solubility and diffusion of the water vapour in the film matrix decreasing it. This behaviour is in agreement with the results of moisture content and solubility. It is interesting the fact that MG-CL films presented higher WVP values than CR-CL for the same $CaCl_2$ concentrations, being in line with the behaviour observed for moisture content, solubility and swelling index. The results showed that it is possible to decrease the WVP values of alginate films through crosslinking which can be useful for food applications where the films are intended to decrease the moisture loss of the food products.

3.3. Swelling index

The non-crosslinked films and MG films crosslinked with 1% (w/v) of $CaCl_2$ are totally soluble and therefore it was not possible to measure the swelling index. For the crosslinked films that maintain their integrity for 24 h, results showed that the swelling index values are reduced for higher $CaCl_2$ concentrations. The effect of $CaCl_2$ concentrations is notable in the CR films (Table 1), being the values for films crosslinked with 1% of $CaCl_2$ around 695.94%. This high swelling index value can be explained by the number of alginate G blocks linked with the calcium ions; in this case the lower concentration of Ca^{2+} leads to the formation of the “egg-box” conformation that is enough to increase the film resistance so they do not dissolve in water, but they still have strands available to uptake water and thus leading to high values of swelling index. The increase of the $CaCl_2$ concentration leads to more bonds between the alginate strands and the Ca^{2+} ions, consequently, alginate chains become less exposed and therefore the swelling index decreases. The MG films showed lower capacity to absorb water when compared with the CR films. In fact, MG films crosslinked with 1% of $CaCl_2$ (MG-CL 1%) were dissolved in water being impossible to measure their swelling index. This can be explained by the lower MW when compared with the CR films, that consequently reduce films strength (Ying, 2006). Also for MG-CL films, the increase of $CaCl_2$ concentration (from 1.25 to 1.5%) leads to low swelling index values. Results showed that M/G ratio have a great influence in the swelling index values. MG films presented the lowest values (281.18% ± 57.45% for 1.25% $CaCl_2$ and 209.99% ± 69.24% for 1.5% $CaCl_2$) when compared with the CR-CL films (485.17% ± 85.14% for 1.25% $CaCl_2$ and 411.54% ± 32.44% for 1.5% $CaCl_2$). Looking at these results, one of the proposals is to use the crosslinked films as pads in meat packaging, acting as absorbers in cuvettes.

3.4. Opacity and colour

Results showed that the film's opacity increased with the crosslinking process for CR and MG films. The crosslinking of the alginate-based films by $CaCl_2$ lead to the formation of stronger bonds resulting in less spaces between the polymer chains and therefore reduces the light that passes through the films and consequently increases opacity. Concerning whiteness (WI), the results showed a difference between CR and MG films probably due to the source of the CR and MG alginates. For MG-CL films the opacity increased for higher concentrations of $CaCl_2$ while for CR-CL films the values were maintained for higher concentrations of $CaCl_2$ (Table 2). In fact, for both types of alginate, the use of $CaCl_2$ concentrations of 1.25% and 1.5% do not lead to significant differences ($p > 0.05$). WI values for the same type of crosslinked alginate film (CR-CL or MG-CL) with different concentrations of $CaCl_2$ (1%, 1.25% and 1.5%) remained constant ($p > 0.05$). The different behaviour between CR and MG films can be explained by the source of alginate and extraction process which can vary according to the type of

Table 2
Values of opacity, whiteness value (WI) and total difference value (ΔE) of CR, MG, CR-CL and MG-CL films crosslinked with 1%, 1.25% and 1.5% of $CaCl_2$.

Film	Opacity (%)	WI	ΔE
CR	$5.38 \pm 2.22^{A,a}$	$59.37 \pm 6.74^{A,a}$	0
CR-CL 1%	$10.34 \pm 1.99^{B,b}$	$71.30 \pm 3.14^{C,b}$	2.59
CR-CL 1.25%	$10.98 \pm 1.68^{C,b}$	$71.14 \pm 7.30^{E,b}$	2.22
CR-CL 1.5%	$11.22 \pm 2.01^{E,b}$	$77.48 \pm 2.50^{F,b}$	3.39
MG	$3.82 \pm 2.45^{A,a}$	$78.35 \pm 4.44^{B,a}$	0
MG-CL 1%	$8.96 \pm 2.70^{B,b}$	$80.97 \pm 3.26^{D,a}$	0.88
MG-CL 1.25%	$12.16 \pm 0.45^{D,c}$	$76.97 \pm 3.07^{E,a}$	0.33
MG-CL 1.5%	$12.10 \pm 0.51^{E,c}$	$80.12 \pm 2.62^{F,a}$	0.43

Values reported are the mean ± sd. Different letters (a–c) in the same column indicate a statistically significant difference ($p < 0.05$) between CR films or MG films and different letters (A-F) indicate a statistically significant difference ($p < 0.05$) between the CR and MG films for the same $CaCl_2$ concentrations.

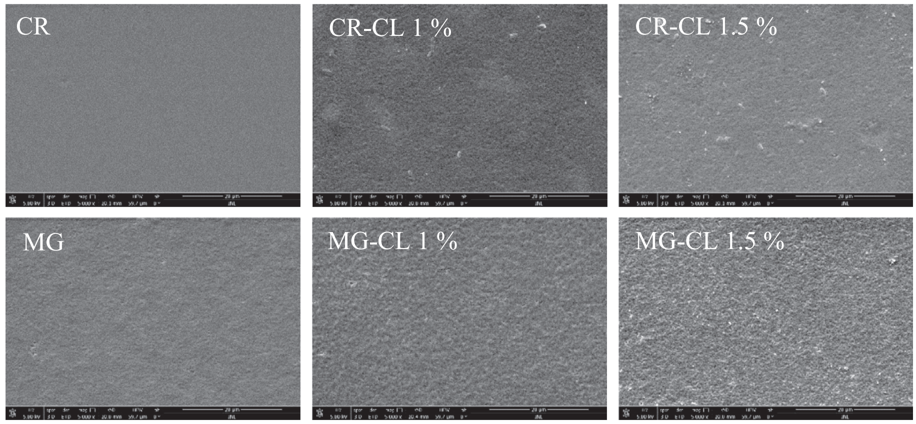

Fig. 2. Surface images obtained by SEM of CR, MG, CR-CL and MG-CL films crosslinked with 1% and 1.5% of $CaCl_2$.

alginate. ΔE shows the differences between the films without crosslinking and crosslinked, being clear the effect of crosslinking in the films' colour parameters. For increasing concentrations of $CaCl_2$ the values are in the same range indicating that the colour of the films remained similar.

3.5. Scanning electron microscopy (SEM)

Fig. 2 shows the surface images of the CR and MG film without crosslinking and CR-CL and MG-CL crosslinked with 1% and 1.5% of $CaCl_2$ obtained by SEM. It is possible to evaluate the surface morphology of the films before and after the crosslinking of CR and MG films with $CaCl_2$. The images show clear differences between CR and MG films where the MG films present higher roughness when compared with CR that presented a smooth surface. It is clear that the roughness and irregularities of the films surface increase when crosslinking occurs and $CaCl_2$ concentrations increase. Crosslinked films presented small aggregate structures that can be explained by the existence of $CaCl_2$ in the film structure and in the “egg-box” structure formed by the interactions between CR or MG alginate structure and the Ca^{2+} ions.

3.6. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy shows the effect of crosslinking in the chemical structure of the MG and CR films. FTIR spectra of CR films and CR-CL films using 1%, 1.25% and 1.5% of $CaCl_2$ (Fig. 3) showed major peaks in the wavenumber ranged between 600 cm^{-1} and 1800 cm^{-1} besides the peaks that are presented between 3700 and 3000 cm^{-1} related to stretching vibration of the O-H bonds (Voo et al., 2015) and between 3000 and 2850 cm^{-1} corresponding to C-H stretching (Lawrie et al., 2007).

The characteristic peaks of alginate were found at 1599 cm^{-1} related to the asymmetric stretching vibration of C-O bond of COO- group (Lawrie et al., 2007; Voo et al., 2015); being the peak at 1408 cm^{-1} related with the symmetric stretching vibration of C-O in COO- group (Pereira, Tojeira, Vaz, Mendes, & Bártolo, 2011; Voo et al., 2015). The peak at 1028 cm^{-1} corresponds to the antisymmetric stretch of C-O-C (Lawrie et al., 2007) and the peak at 818 cm^{-1} is characteristic of mannuronic acid residues (Fertah, Belfkira, Dahmane, Taourirte, & Brouillette, 2017). For CR films, the peak at 1599 cm^{-1} underwent a shift to 1595 cm^{-1}, showing the involvement of COO- group in the crosslinking process (Voo et al., 2015).

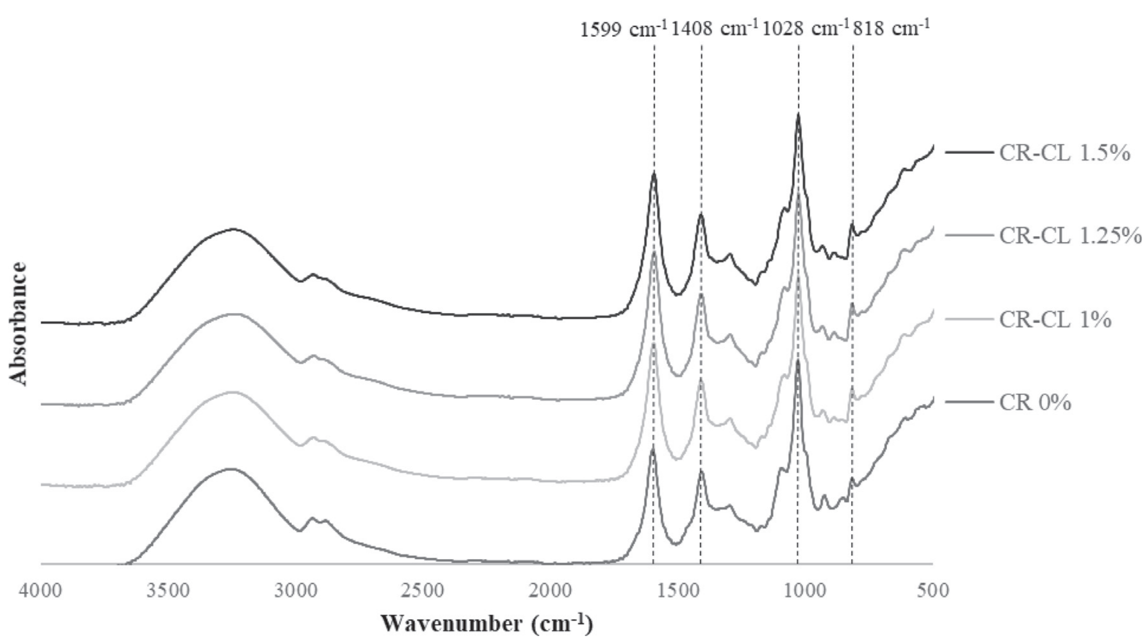

Fig. 3. FTIR spectra of CR films without (CR 0%) and with crosslinking ($CaCl_2$ – 1%, 1.25% and 1.5%) - (CR-CL 1%, CR-CL 1.25% and CR-CL 1.5%).

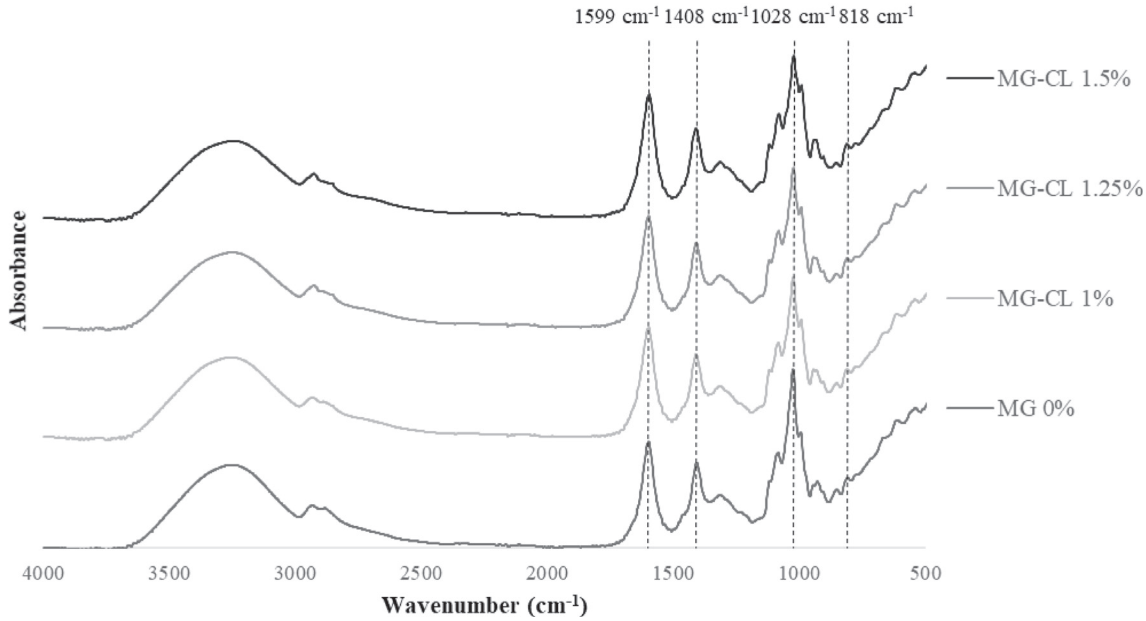

Fig. 4. FTIR spectra of MG films without (MG 0%) and with crosslinking ($CaCl_2$ − 1%, 1.25% and 1.5%) - (MG-CL 1%, MG-CL 1.25% and MG-CL 1.5%).

Fig. 4 shows the FTIR spectra for MG films crosslinked with different concentrations of $CaCl_2$. The band between 800 and 820 cm^{-1} is related to mannuronic acid residues and, it is located between 810 and 814 cm^{-1} in MG films, while, it is located in the 818 cm^{-1} region for CR films; this is explained by the high M content of CR alginate compared to MG alginate. MG and CR films also showed bands between 810 and 930 cm^{-1}, which are related to polymannuronic and polyguluronic sequences in the alginate, and bands in the region between 476 cm^{-1} and 607 cm^{-1}, which are related to the vibrational modes of pyranose rings (Singh, Sharma, & Chauhan, 2010). Changes were observed in these two spectra regions between the films prepared with CR alginate and MG alginate, indicating that crosslinking had a different effect on the alginate backbone structure as a function of the alginate type. For CR-CL films there are minor differences between films crosslinked with different concentrations of $CaCl_2$, while for MG-CL films there is a clear difference when the $CaCl_2$ concentrations are increased. This behaviour indicates a crosslinking saturation point for CR-CL films, in agreement with the results obtained for moisture, solubility and swelling index.

3.7. Mechanical properties

Table 3 shows the values of elongation-at-break (EB), tensile strength (TS) and Young's modulus (YM) of the alginate-based films. Results showed that TS and YM values increased for higher concentrations of $CaCl_2$, while EB values decreased. CR films showed values of 9.28 ± 2.74 MPa, while CR-CL films reached values of 38.74 MPa (1.5% $CaCl_2$). For CR films, EB values decreased from 38.88% to 6.22% (1.5% $CaCl_2$) and no statistical differences were observed between the crosslinked films ($p > 0.05$). Also, YM values increased from 0.23 MPa in CR films to 14.13 MPa in CR-CL films crosslinked with 1.5% $CaCl_2$. The same behaviour was observed for MG films, but the obtained values of TS and EB are lower than the ones obtained for CR films. This difference is due to the type of alginate, since MG has a different M/G ratio and presents a lower molecular weight (<200 kDa) than CR (300 kDa) (Lee & Mooney, 2012).

The higher TS and YM values obtained for the crosslinked films indicate an increase of the film resistance and stiffness, which is explained by the reaction of alginate with Ca^{2+}, and consequently the formation of the "egg-box" structure. These results are explained by fewer H_2O molecules bind to the alginate structure, as explained previously (moisture content section), which reduces the free volume in the films structure and leads to the increase of TS and the decrease of EB in both CR-CL and MG-CL films. Therefore, the enhancement of the bonds established between the polymer chains and Ca^{2+} ion that increase with the concentration of $CaCl_2$ solution leads to stronger and more cohesive films and consequently less flexible. This behaviour is in agreement with published results (Pavlath, Voisin, & Robertson, 1998; Rhim, 2004).

Table 3
Values of elongation-at-break, tensile strength and Young's Modulus of CR, MG and CR-CL and MG-CL films crosslinked with 1%, 1.25% and 1.5% of $CaCl_2$.

Film	Elongation-at-break (%)	Tensile strength (MPa)	Young's Modulus (MPa)
CR	$38.88 \pm 2.23^{A,a}$	$9.28 \pm 2.74^{A,a}$	$0.23 \pm 0.06^{A,a}$
CR-CL 1%	$7.43 \pm 1.14^{C,b}$	$27.22 \pm 2.84^{C,b}$	$10.69 \pm 1.78^{C,b}$
CR-CL 1.25%	$5.47 \pm 2.11^{D,b}$	$31.00 \pm 6.76^{D,b,c}$	$12.44 \pm 2.10^{D,bc}$
CR-CL 1.5%	$6.22 \pm 0.82^{F,b}$	$38.74 \pm 5.59^{F,c}$	$14.13 \pm 2.14^{E,c}$
MG	$21.24 \pm 1.43^{B,a}$	$3.72 \pm 1.25^{B,a}$	$0.16 \pm 0.01^{B,a}$
MG-CL 1%	$6.81 \pm 0.50^{C,b}$	$25.92 \pm 2.89^{C,b}$	$11.51 \pm 1.45^{C,a}$
MG-CL 1.25%	$2.39 \pm 0.38^{E,c}$	$19.48 \pm 2.98^{E,c}$	$12.19 \pm 1.23^{D,b}$
MG-CL 1.5%	$2.93 \pm 0.43^{G,c}$	$26.43 \pm 4.76^{G,b}$	$14.90 \pm 2.49^{E,c}$

Values reported are the mean ± sd. Different letters (a–c) in the same column indicate a statistically significant difference ($p < 0.05$) between CR films or MG films and different letters (A-G) indicate a statistically significant difference ($p < 0.05$) between the CR and MG films for the same $CaCl_2$ concentrations.

4. Conclusion

Alginate-based films are influenced by their M/G ratio and molecular weight, and the crosslinking process can be used to change their main properties. Films crosslinked with $CaCl_2$ (both for CR and MG films) present higher tensile strength values and lower values of elongation-at-break, as well as low MC, solubility and swelling index values. Different concentrations of crosslinker influenced films' properties according to the alginate type, being possible to conclude that for CR films a crosslinking saturation point was achieved, while for the MG films higher concentrations of $CaCl_2$ are needed to obtain similar properties. This is explained by the high G content of MG alginate. On the other side the higher amount of mannuronic acid in relation to the guluronic acid leads to stronger and less soluble films (that is the case of CR films). Results obtained here will help the processing and optimization of alginate films', since now the interactions that occur between alginates with different M/G ratio and different molecular weight with different concentrations of $CaCl_2$ are better understood.

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